Nonclassical activation of gli1 as a therapeutic target for squamous cell lung cancer

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Background: The Hedgehog (Hh) signaling pathway is critical for embryonic development and its deregulation is implicated in a number of tumor types. The role of the Hh signaling pathway, however, in the initiation and growth of non-small cell lung cancer is largely unknown. Here, we investigate the role of the Hh pathway transcription factor, GLI1, in lung squamous cell carcinoma (SCC) and as a potential therapeutic target for treatment of lung SCC.

Methods: GLI1 expression in human SCC cell lines was evaluated by quantitative PCR and Western Blot. siRNA and shRNA of GLI1 in these cell lines were utilized in vitro and in vivo to test the requirement of GLI1 in tumor growth. Small molecule modulators of GLI1 were tested for their therapeutic potential.

Results: GLI1 mRNA expression was significantly elevated in lung SCC compared to normal lung and lung adenocarcinoma patient specimens in several human genomic databases. Importantly, overexpression of GLI1 was correlated with poor overall survival in lung cancer patients. siRNA-mediated knock down of GLI1 in SCC cell lines decreased the expression of GLI1 target genes and caused a significant reduction in colony formation. Stable knock down of GLI1 in SCC cell lines caused a significant reduction in growth of xenograft tumors indicating the critical role of GLI1 in lung SCC progression. Inhibition or activation of SMO, an upstream component of Hh pathway, did not alter GLI1 expression level in lung SCC cell lines. However, inhibition of PI3K/AKT and MAPK signaling pathways down-regulated GLI1 expression, suggesting that GLI1 expression is dependent on PI3K/AKT and MAPK pathway activity rather than Hh ligand. Small molecule inhibition of PI3K/mTOR pathway or GLI1 significantly reduced GLI1 expression, proliferation, and clonogenicity in SCC cell lines. Combinatorial inhibition of PI3K and GLI1 by BKM120 and arsenic trioxide (ATO), respectively, significantly abrogated the in vivo growth SCC tumors in mice and correlated with decreased tumor GLI1 expression.

Conclusion: Our findings demonstrate that GLI1 is essential for lung SCC tumor progression. Furthermore, GLI1 expression in SCC is independent of Hh pathway ligand action and dependent on MAPK and PI3K pathway activity. Direct inhibition of GLI1 by repurposing ATO in combination with a PI3K inhibitor may represent a novel therapeutic strategy for lung SCC.

Validation of L-Myc as a viable therapeutic target in small cell lung cancer

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Background and Hypothesis: The paucity of molecular targets for small cell lung cancer (SCLC) chemoprevention and therapy is largely due to the poor understanding of SCLC progression beyond the role of RB and P53 mutations crucial for tumor initiation. Amplification of the Myc family of oncogenes is one of the most frequent alterations in human SCLC genomes. However, the concept of inhibiting these factors to intervene in SCLC progression, despite its clear value as a targeted therapy, has not been formally tested in the autochthonous model. We tested the hypothesis that L-Myc is a key determinant of SCLC and its pathway as a viable target for therapeutics and chemoprevention.

Methods and Results: Using comparative gene expression analysis of pre-cancerous cells (preSC) and tumor cells, both derived from the genetically engineered mouse model (GEMM), we identified a gene set specific to SCLC tumorigenic progression and found that L-Myc is the most up-regulated gene in the mouse model. Retroviral overexpression of L-Myc, mimicking the gene amplification, was sufficient to cause the tumorigenic progression of the L-Myc-expressing preSC in culture or allograft experiment, while CRISPR-mediated knockout of L-Myc blocked the long-term growth of SCLC cells in culture. Comparison of L-Myc-preSC with control (non-transformed) preSC revealed a specific gene signature, and the pathway analysis of the signature indicated significant activation of several molecular pathways, including epithelium-to-mesenchyme transition, downstream of L-Myc during tumor progression. More significantly, conditional deletion of L-Myc in the GEMM dramatically reduced tumor burden in a